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1636

TRANSMITTAL LETTER (General - Patent Pending)	Docket No. 16365Z
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In Re Application Of: Tom A. Grigliatti, et al.

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/896,888	June 29, 2001	Nancy S. Vogel	23389	1636	3346

Title: INSECT EXPRESSION VECTORS


COMMISSIONER FOR PATENTS:

Transmitted herewith is:

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- Certified Copy of Canadian Patent Application No. CA 2221819
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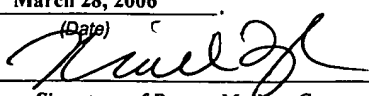
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Dated: March 28, 2006

Xiaochun Zhu  
Registration No. 56,311  
Scully, Scott, Murphy & Presser, P.C.  
400 Garden City Plaza-STE 300  
Garden City, New York 11530  
(516) 742-4343

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**Applicants:** Tom A. Grigliatti, et al.                      **Examiner:** Nancy S. Vogel  
**Serial No.:** 09/896,888                      **Art Unit:** 1636  
**Filed:** June 29, 2001                      **Docket:** 16365Z  
**For:** INSECT EXPRESSION VECTORS                      **Dated:** March 28, 2006

**Confirmation No.:** 3346


Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

**CLAIM OF PRIORITY**

**Sir:**

Applicants in the above-identified application hereby claim the right of priority to Canadian Patent Application No. CA 2221819, filed on January 28, 1998 under 35 U.S.C. §119. Applicants submit herewith a certified copy of CA 2221819.

Respectfully submitted,

  
Xiaochun Zhu  
Registration No. 56,311

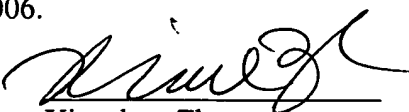
Scully, Scott, Murphy & Presser, P.C.  
400 Garden City Plaza-STE 300  
Garden City, New York 11530  
(516) 742-4343  
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Specification and Drawings, as originally filed, with Application for Patent Serial No:  
**CA 2221819** on January 28, 1998 by **THE UNIVERSITY OF BRITISH COLUMBIA**,  
assignee of Thomas A. Gigliatti, Dave A. Theilmann, Thomas A. Pfeifer and Dwayne D.  
Hegedus, for "Insect Expression Vectors"


*Sylvie Grogan*  
Agent certificateur/Certifying Officer

March 10, 2006

Date

Canada

(CIPO 68)  
31-03-04

OPIC  CIPO

## **ABSTRACT**

The invention provides insect shuttle vectors, and methods of using such vectors, for stably transforming disparate insect cell lines to express heterologous proteins. The invention provides a transformed insect cell selection system based on resistance to the bleomycin/phleomycin family of antibiotics, including the antibiotic Zeocin. Efficient promoters derived from baculovirus immediate early promoters are disclosed for use in directing expression of heterologous proteins, including selectable markers, in transformed insect cells of the invention. Transposon-based vectors are disclosed that provide inducible transposition to optimize heterologous protein expression and unobtrusive markers to facilitate selection of desired transformants.

**What is claimed is:**

1. A shuttle vector for transforming insect cells, comprising:
  - a. a prokaryotic origin of replication;
  - 5 b. an insect promoter having homology to, and capable of functioning as, an immediate early baculovirus promoter;
  - c. a prokaryotic promoter sequence;
  - d. a selectable marker gene capable of conferring resistance to a  
10 bleomycin/phleomycin-type antibiotic under the transcriptional control of the insect promoter and the prokaryotic promoter sequence, in insect and prokaryotic cells respectively.
- 15 2. The shuttle vector of claim 1, wherein the prokaryotic promoter sequence is a cryptic promoter within the insect promoter.
3. The shuttle vector of claim 1, wherein the bleomycin/phleomycin-type antibiotic is Zeocin.
4. The shuttle vector of claim 1, further comprising an insertion site for heterologous DNA.  
20
5. The shuttle vector of claim 4, wherein the insertion site for heterologous DNA is under the transcriptional control of a second insect promoter.

6. The shuttle vector of claim 5, further comprising a heterologous DNA sequence inserted at the insertion site and under the transcriptional control of the second insect promoter.

7. The shuttle vector of claim 1, wherein the insect promoter comprises an IE2B element substantially homologous to SEQ ID NO: 10.

8. The shuttle vector of claim 7, wherein the insect promoter comprises a GATA-IE2B element pair substantially homologous to SEQ ID NO: 9 and SEQ ID NO: 10.

9. The shuttle vector of claim 8, wherein the insect promoter comprises a sequence substantially homologous to SEQ ID NO: 1 from bp 351 to bp 527.

10. The shuttle vector of claim 9, wherein the insect promoter comprises a sequence substantially homologous to SEQ ID NO: 1.

11. The shuttle vector of claim 1 further comprising DNA transposable elements defining a transposon.

12. The shuttle vector of claim 11, wherein the selectable marker gene is within the transposon.

13. The shuttle vector of claim 12, further comprising an insertion site for heterologous DNA within the transposon.
14. The shuttle vector of claim 13, further comprising a heterologous DNA sequence inserted at the insertion site and under the transcriptional control of a second insect promoter.
15. The shuttle vector of claim 11, further comprising an inducible transposase gene within the transposon.
16. Insect cells transformed with the shuttle vector of claim 1.
17. Insect cells transformed with the shuttle vector of claim 11.
18. A method of transforming insect cells comprising:
- a. inducing the insect cells to take up an insect shuttle vector comprising a selectable marker gene under the transcriptional control of an insect promoter, the selectable marker gene being capable of conferring resistance to a bleomycin/phleomycin-type antibiotic, and the insect promoter having homology to, and being capable of functioning as, an immediate early baculovirus promoter; and,
  - b. selecting transformed cells that are resistant to the bleomycin/phleomycin-type antibiotic.

19. A method of increasing the copy number of a heterologous DNA sequence in a recombinant cell, comprising:
- a. providing a recombinant cell having,
    - i. the heterologous DNA sequence positioned within a transposon defined by DNA transposable elements;
    - ii. an unobtrusive marker gene linked to the heterologous DNA sequence within the transposon;
    - iii. a transposase gene, the transposase being capable of mediating replicative transposition of the transposon;
  - b. permitting expression of the transposase gene to mediate replicative transposition of the heterologous DNA sequence; and,
  - c. monitoring the increase in copy number of the heterologous DNA sequence by monitoring the expression of the unobtrusive marker gene.
20. The method of claim 19 wherein the transposase gene is inducible and the step of permitting expression of the transposase gene comprises inducing expression of the transposase gene.
21. Recombinant insect cells transformed with the shuttle vector of claim 1, expressing a heterologous protein selected from the group consisting of melanotransferrins and biologically active derivatives thereof.

22. A heterologous protein produced by the cells of claim 21, selected from the group consisting of melanotransferrins and biologically active derivatives thereof.
23. Recombinant insect cells transformed with the shuttle vector of claim 1, expressing a heterologous protein selected from the group consisting of insect ion transport peptide hormones and biologically active derivatives thereof.
24. A heterologous protein produced by the cells of claim 23, selected from the group consisting of insect ion transport peptide hormones and biologically active derivatives thereof.
25. Insects comprising cells stably transformed with the vector of claim 1.
26. A recombinant cell comprising a heterologous, inducible transposase gene, wherein transposase is expressed in the cell at levels that mediate transposition only upon induction of the transposase gene.

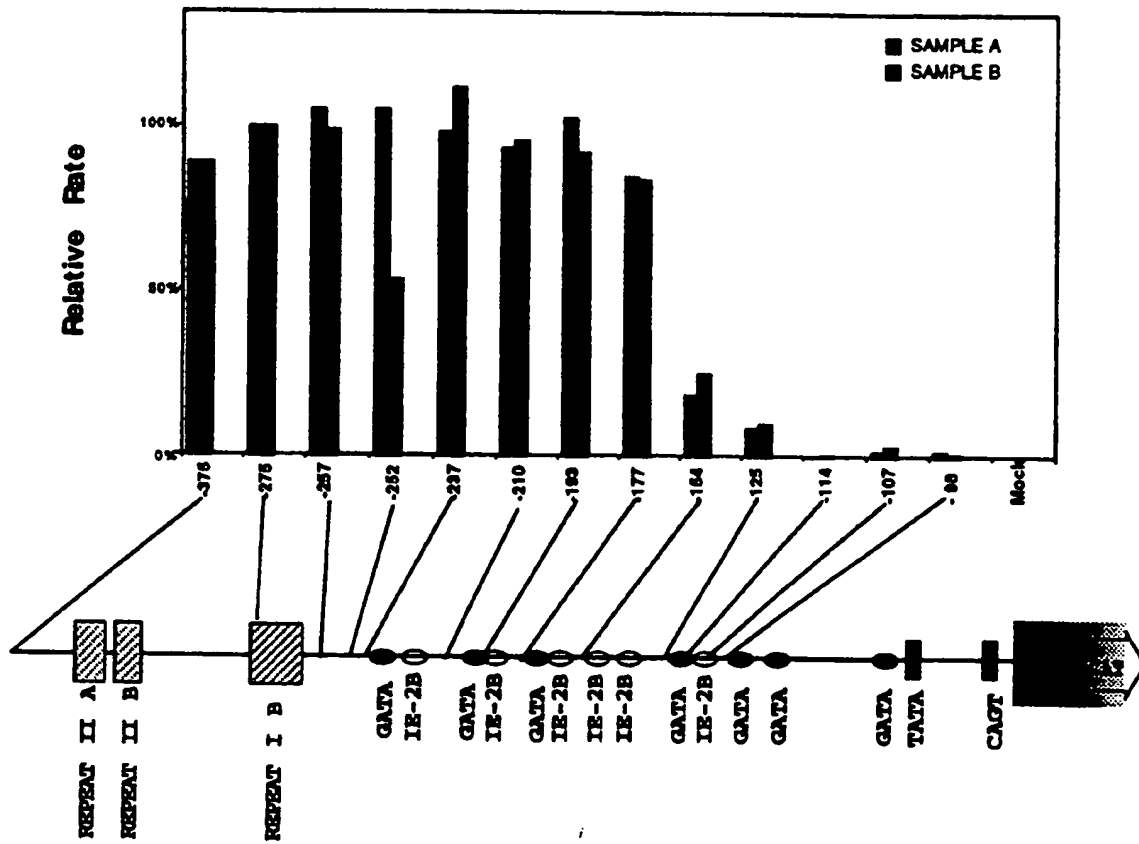


Figure 1

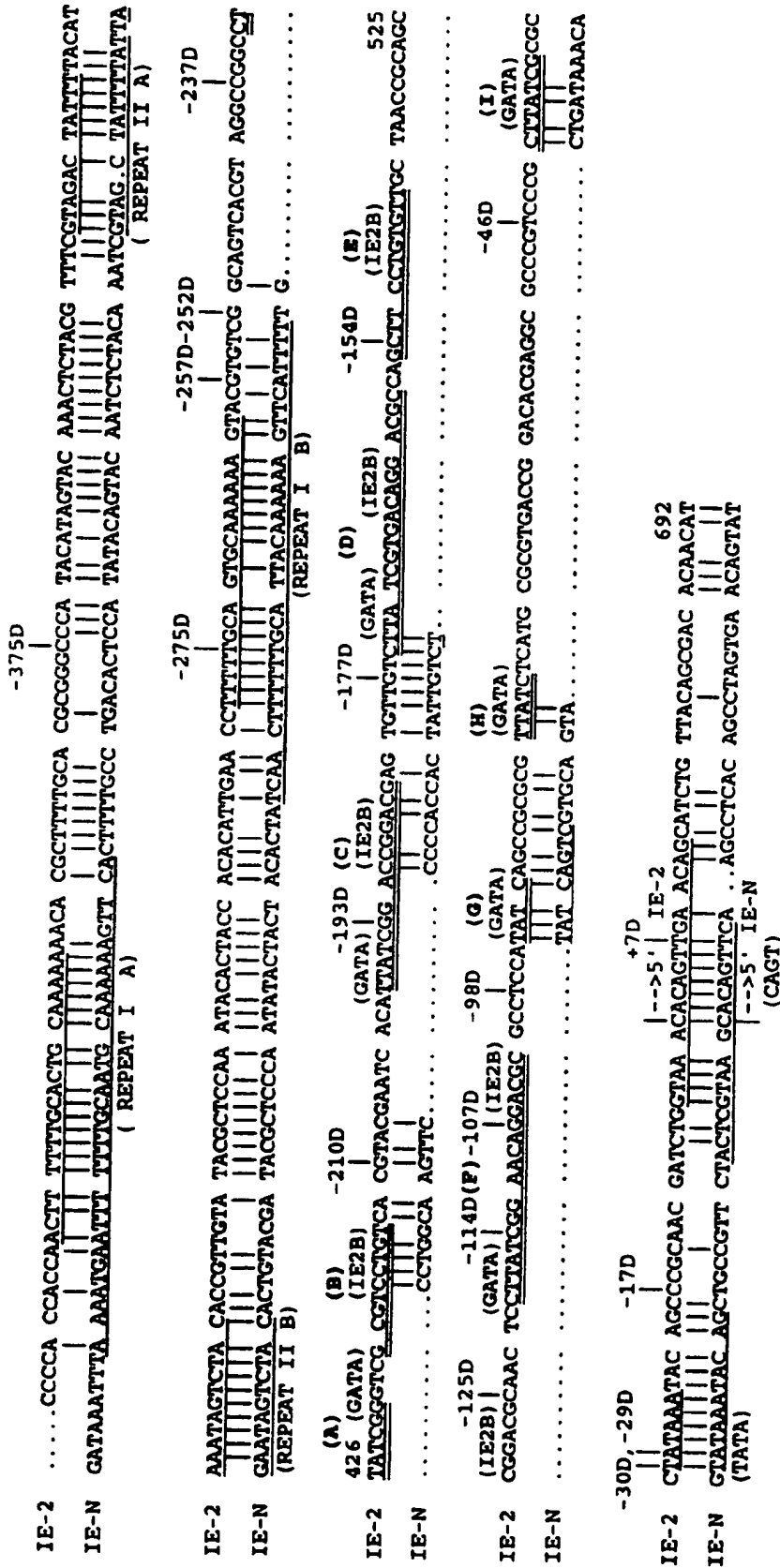


Figure 2a



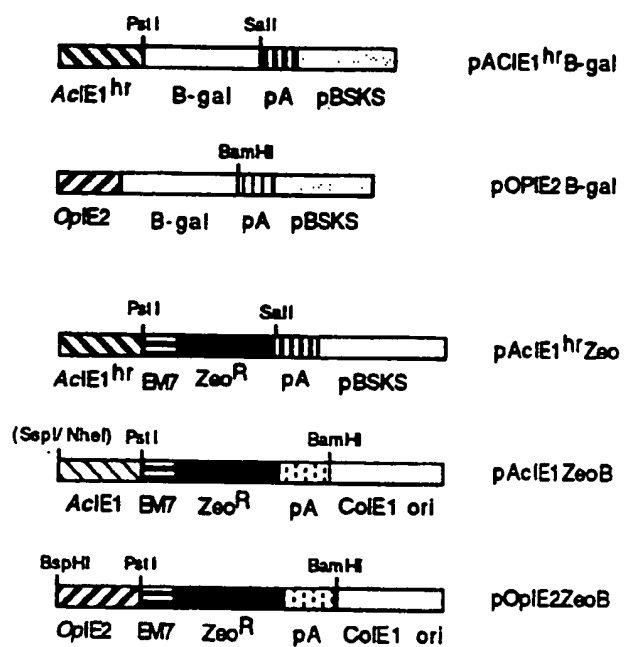


Figure 3

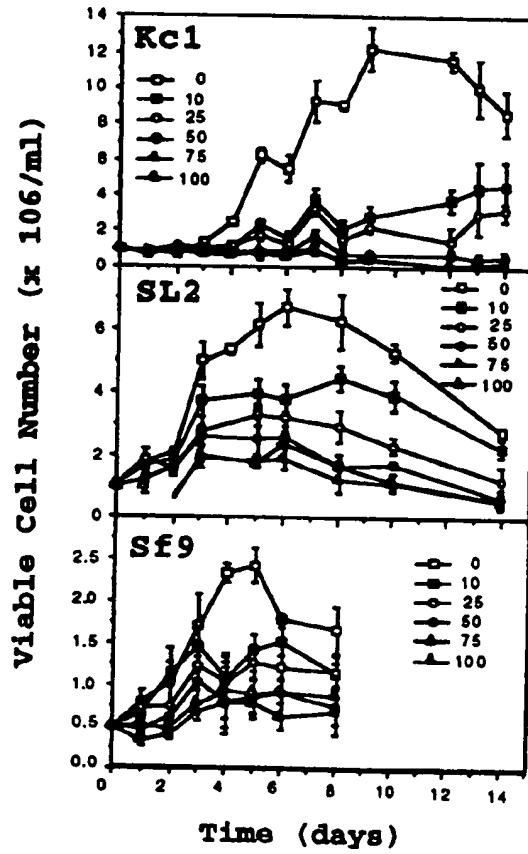


Figure 4a

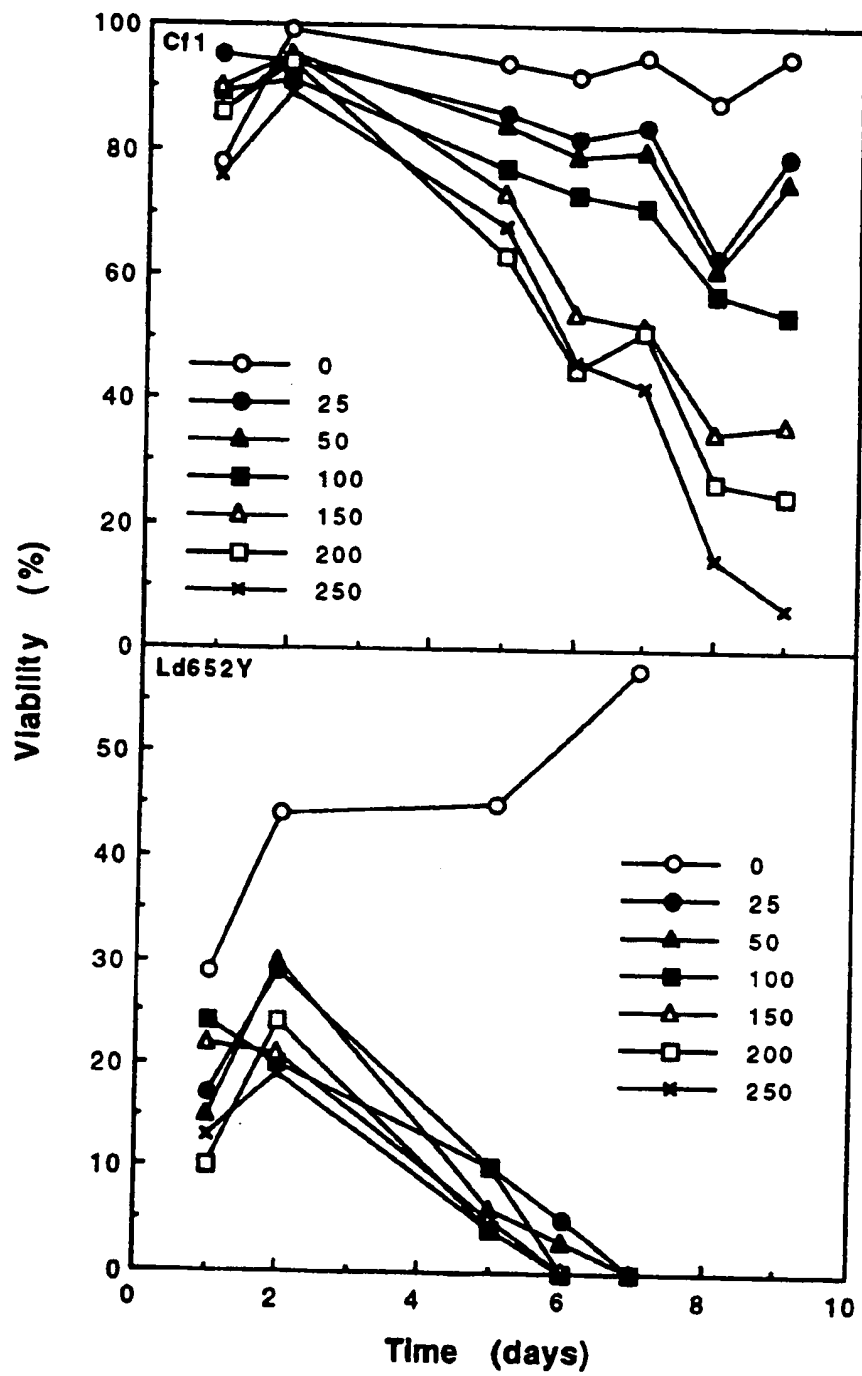


Figure 4b

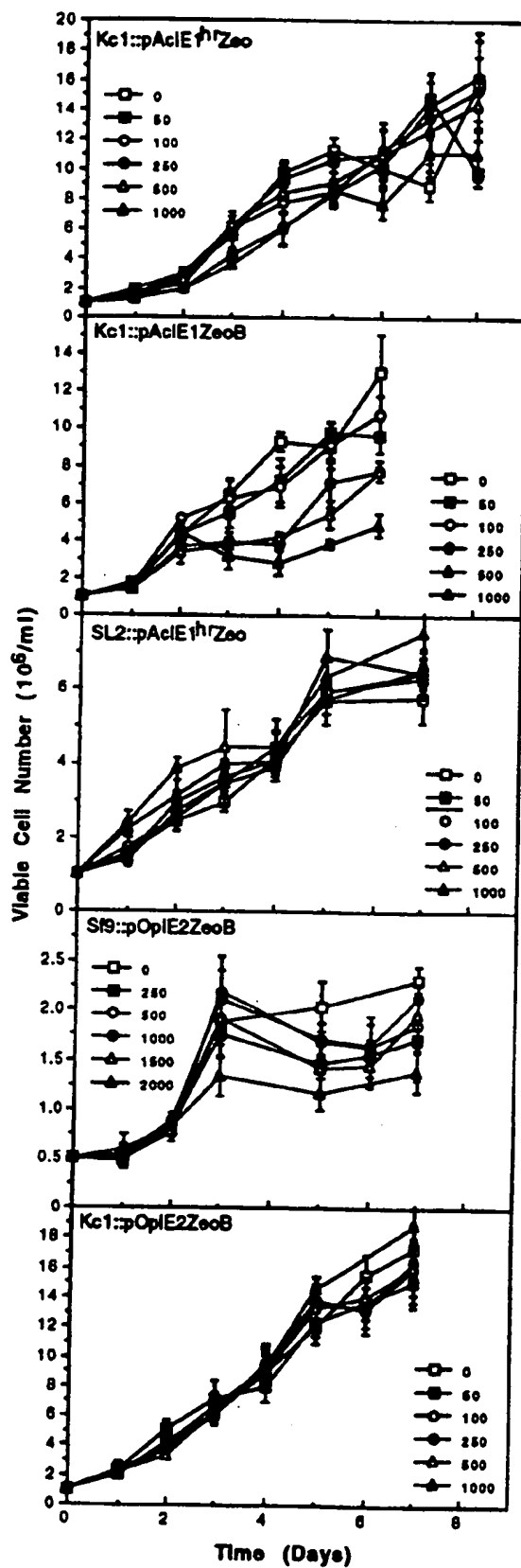


Figure 5

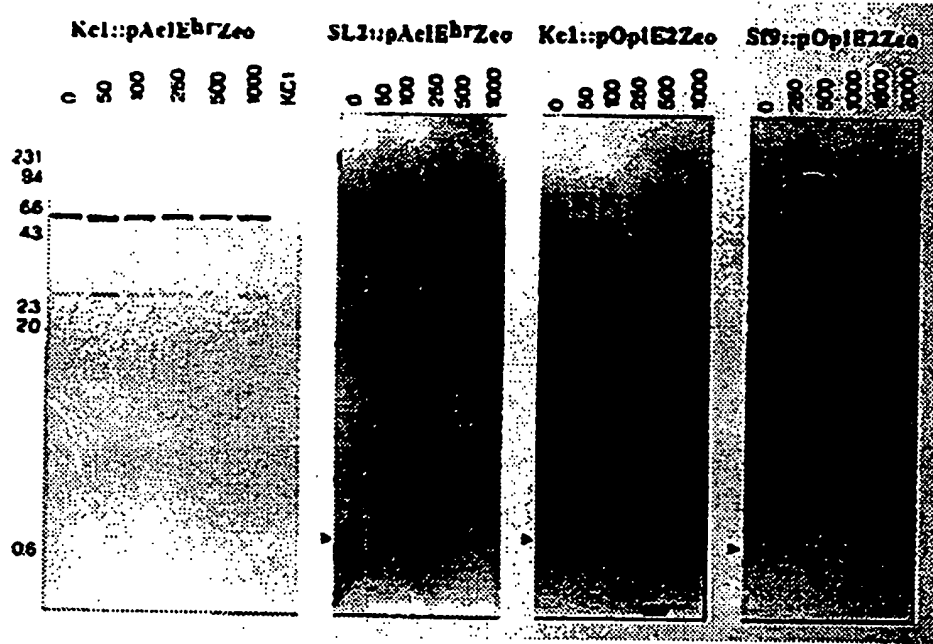
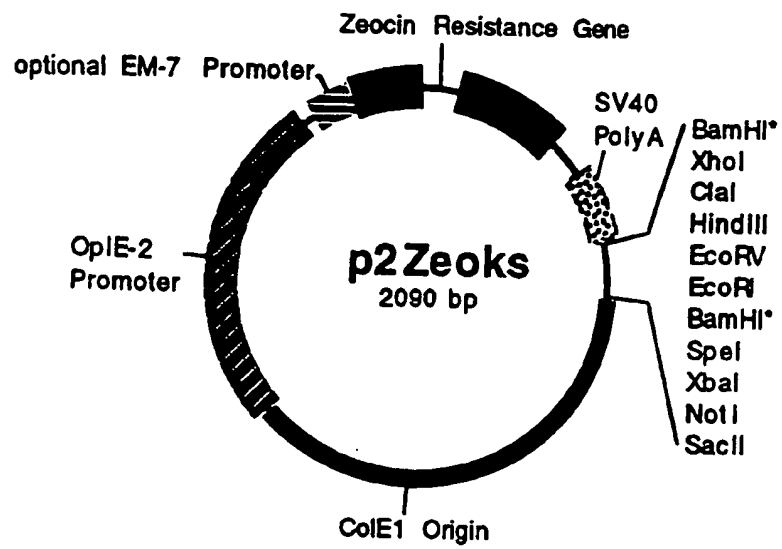


Figure 6



**Figure 7**

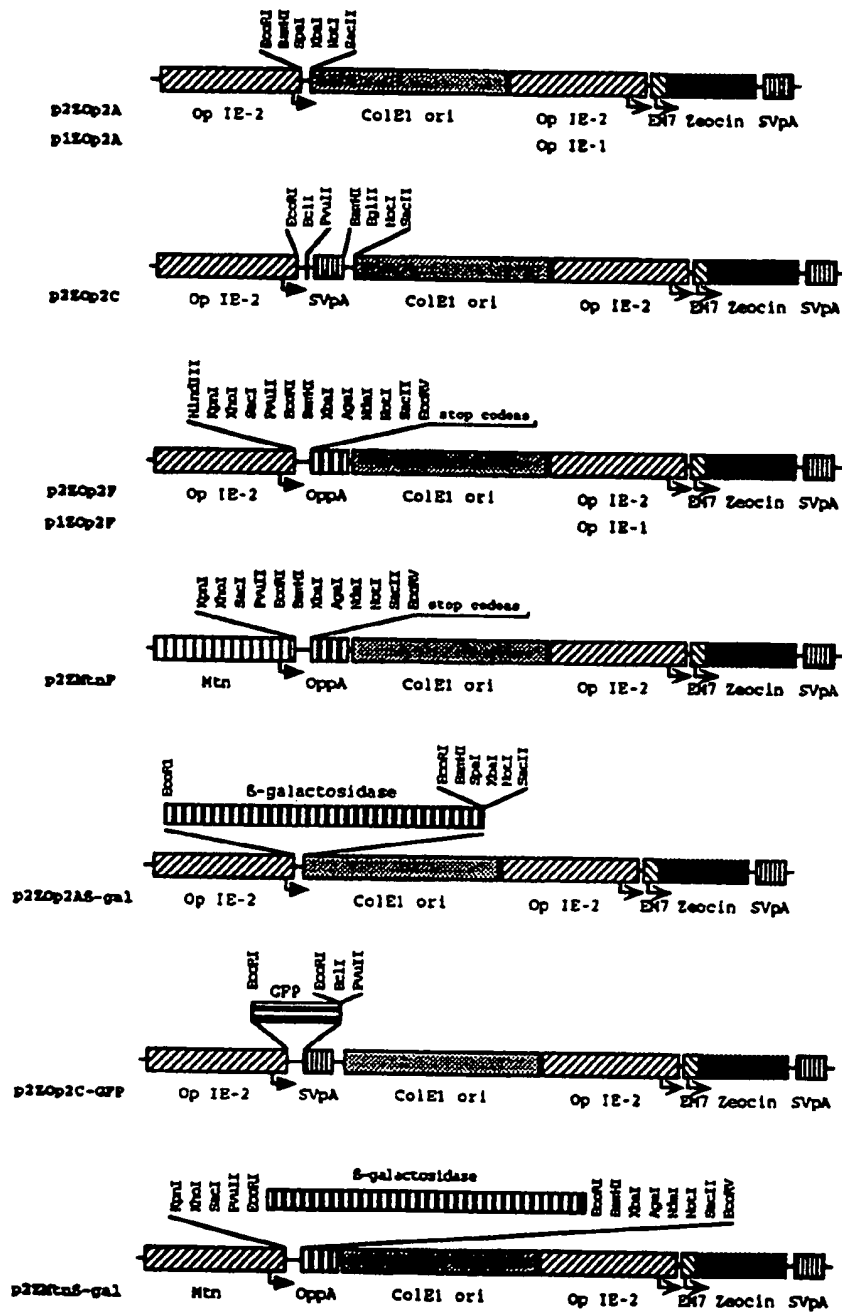


Figure 8

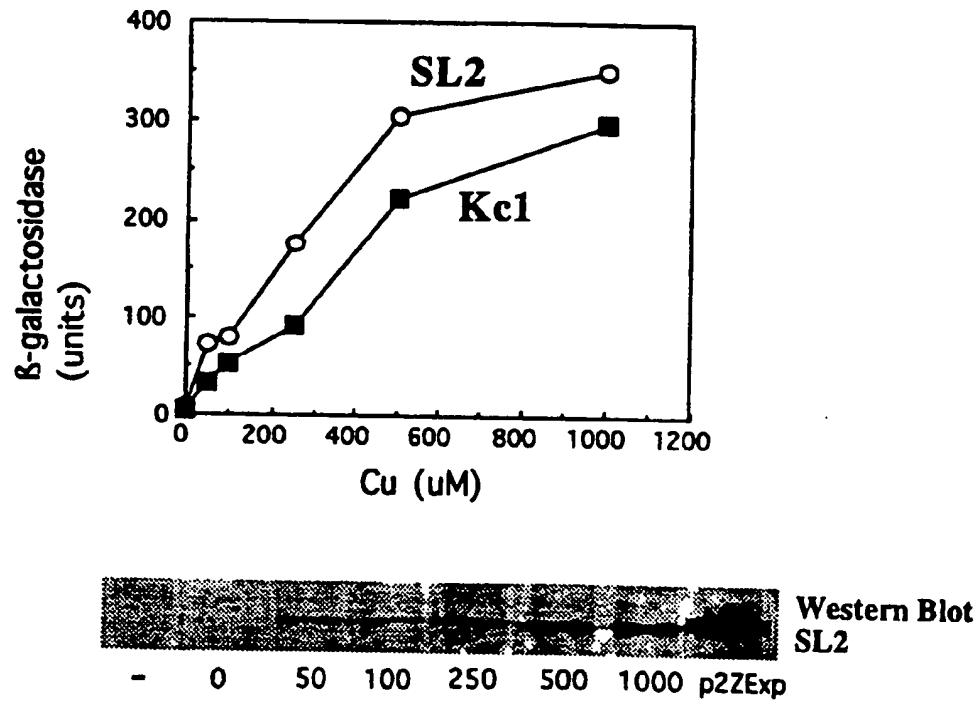


Figure 9

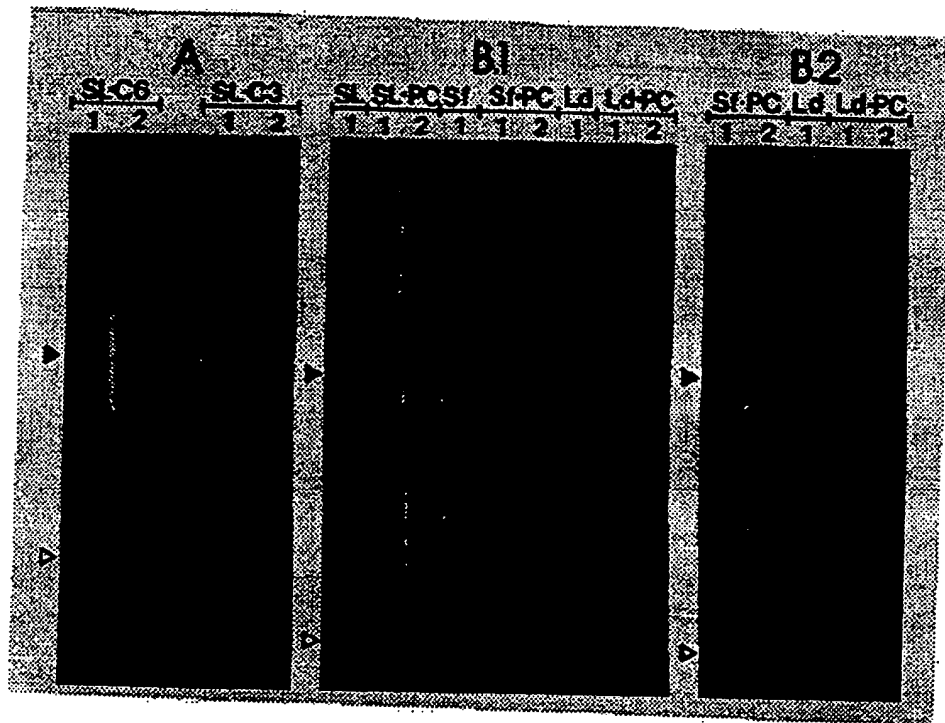


Figure 10

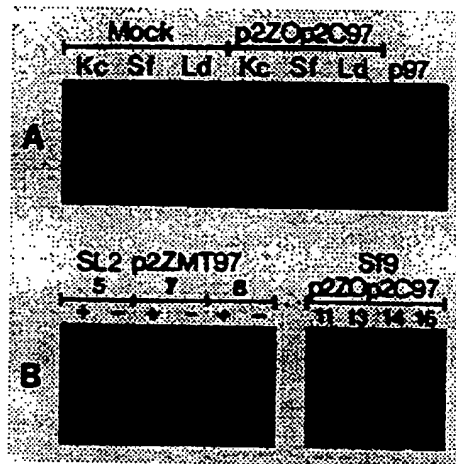
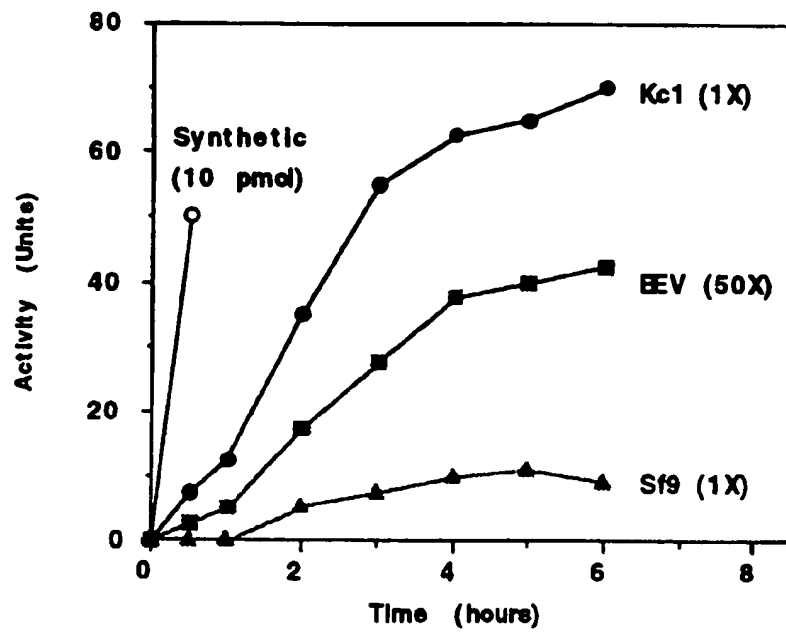


Figure 11



**Figure 12**

**Figure 13**

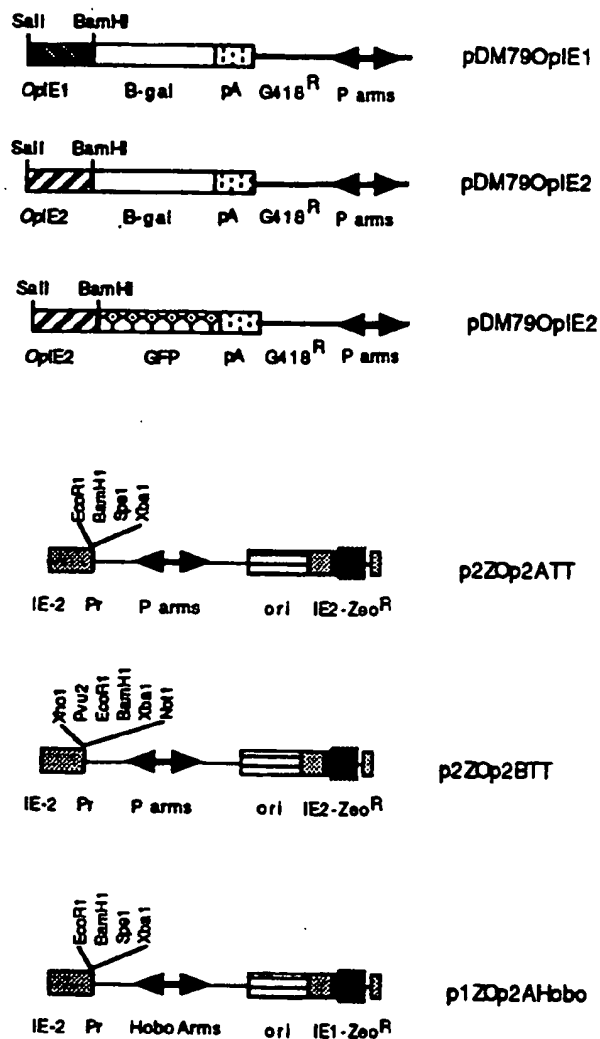


Figure 14

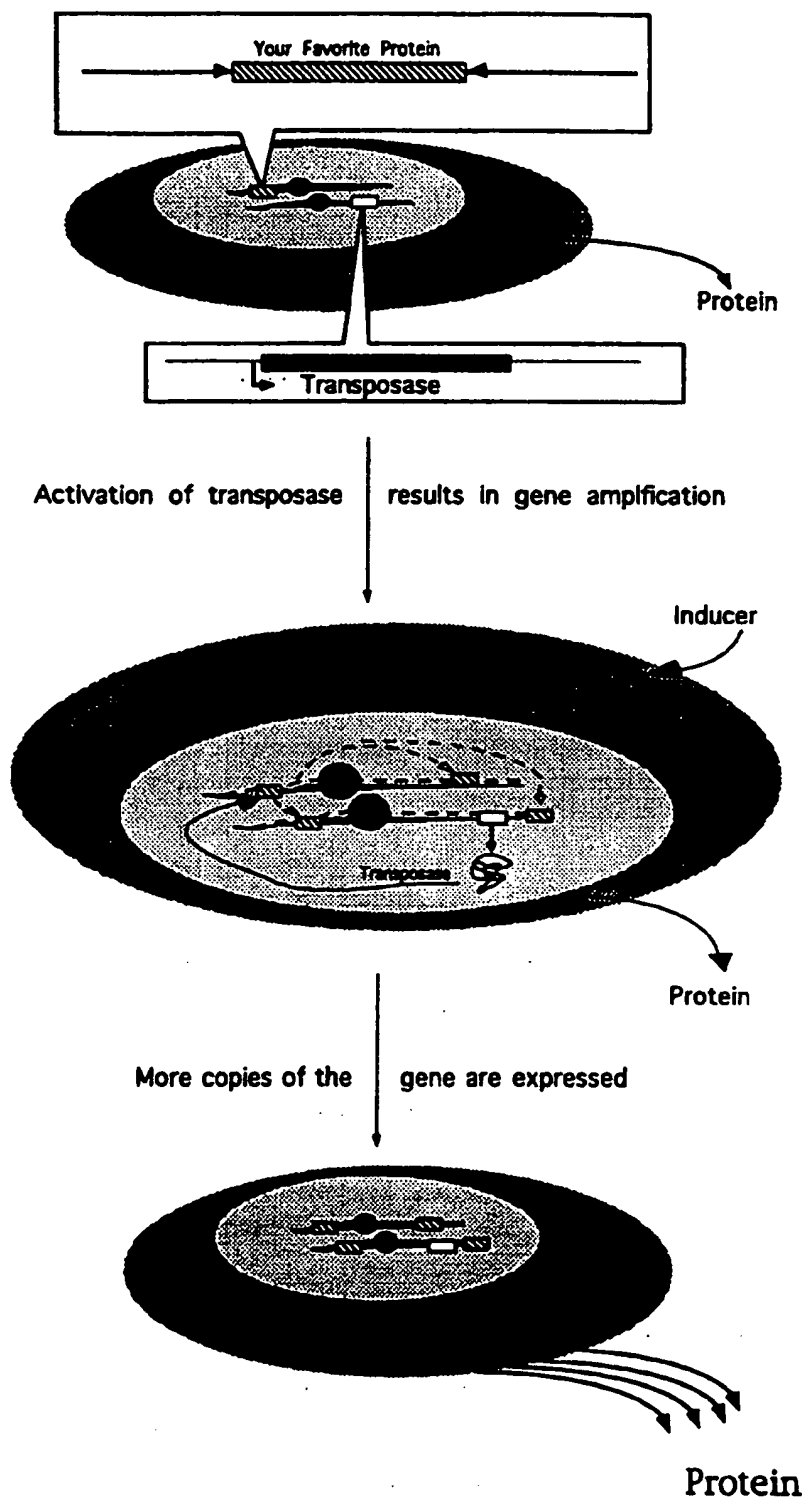
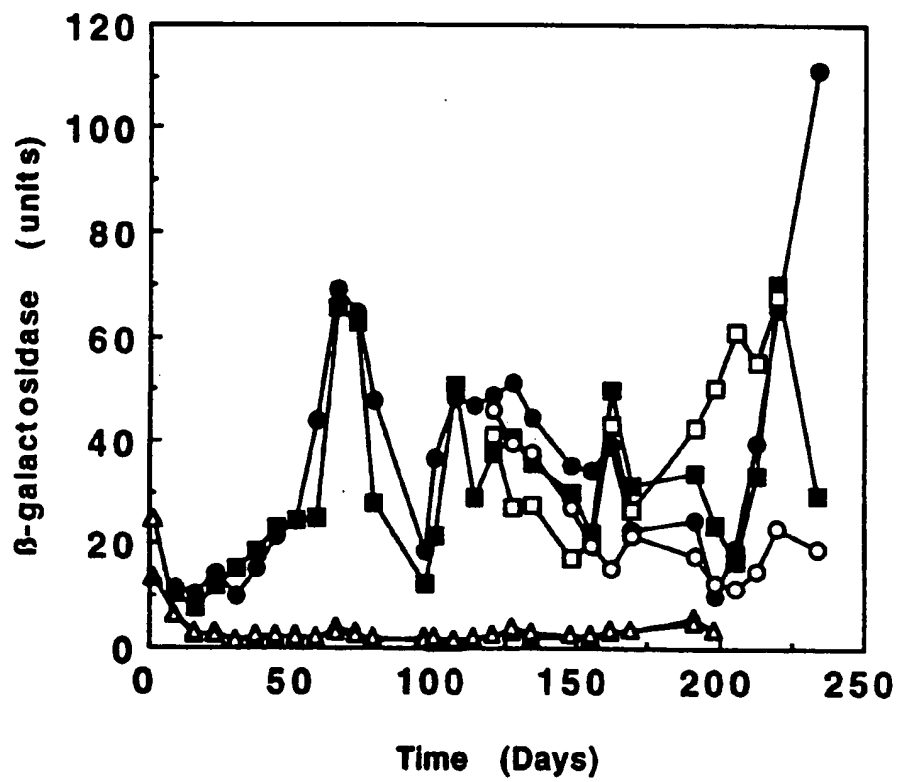


Figure 15



**Figure 16**

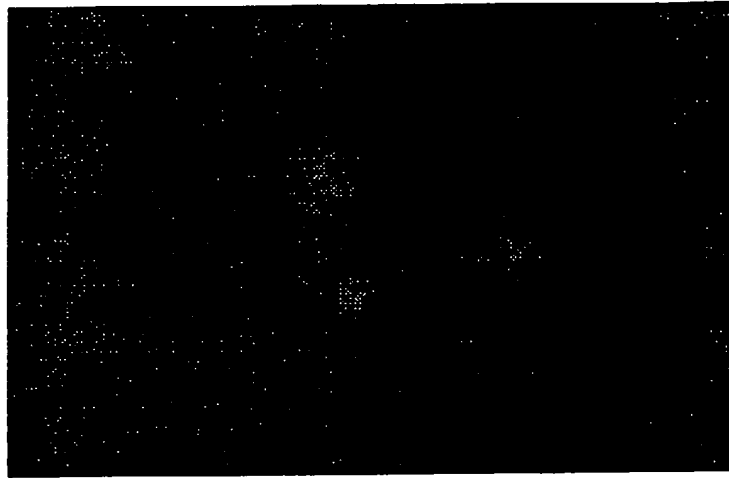


Figure 17

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